PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 29 March 2001 (29.03.01)	
International application No. PCT/US00/19336	**Applicant's or agent's file reference** 4152-3-PCT
International filing date (day/month/year) 13 July 2000 (13.07.00)	**Priority date** (day/month/year) 13 July 1999 (13.07.99)
Applicant COX, George, N., III et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 January 2001 (08.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer S. Mafla
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4152-3-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/19336	International filing date *(day/month/year)* 13 July 2000 (13.07.2000)	Priority date *(day/month/year)* 13 July 1999 (13.07.1999)
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 39/395, 39/00; C07K 16/00, 01/00 and US Cl.: 424/134.1, 185.1, 192.1; 435/69.7; 530/387.3, 351		
Applicant BOLDER NIOTECHNOLOGY INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☑ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08 January 2001 (08.01.2001)	02 October 2001 (02.10.2001)
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Jessica H. Roark Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/19336

I. Basis f the report

1. With regard to the elements of the international application:*

☒ the international application as originally filed.

☒ the description:
pages 1-62 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages NONE, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages 63-65, filed with the letter of 15 August 2001 (15.08.2001)

☒ the drawings:
pages 1, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*
*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/19336

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 6-14,22,23 and 26-37

because:

☐ the said international application, or the said claim Nos. ____ relate to the following subject matter which does not require international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 6 are so unclear that no meaningful opinion could be formed *(specify)*:

Claim 6 is an improper multiple dependent claim as per PCT Rule 6.4(a).

☐ the claims, or said claims Nos. ____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 7-14, 22-23 and 26-37

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	3-5 and 17-20 and 25	YES
	Claims	1-2, 15-16, 21 and 24	NO
Inventive Step (IS)	Claims	17-20 and 25	YES
	Claims	1-5, 15-16, 21 and 24	NO
Industrial Applicability (IA)	Claims	1-5, 15-21 and 24-25	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Section I. Basis of the report, Item 5
The amendment of the claims, filed 15 August 2001 is objected to under PCT Article 34(2)(b) because it adds matter into the application that goes beyond the disclosure as orginally filed. The added matter which appears to be new is as follows:

The amendment filed 15 August 2001 does not point to a passage in the disclosure for the added matter. In claim 40, both the lower limitation of "two or more" and the negative limitation of "wherein the peptide linker is not Gly 3-7" appear to be added matter that goes beyond the disclosure as originally filed.

1. Claims 1-2 and 24 lack novelty under PCT Article 33(2) as being anticipated by US 5,650,150 (GILLIES).

US 5,650,150 teaches a fusion protein comprising a the cytokines IL-2, lymphotoxin, or GM-CSF joined either directly or via a linker sequence comprising a proteolytic cleavage site to an immunoglobulin domain (see entire document, especially claims 1, 3 and 7-11). In addition, a purified dimeric Ig fusion protein is taught, since the CH3-LT fusion protein forms dimers (e.g., column 8, especially lines 56-65), and can be purified (e.g., column 9, lines 25-43).

2. Claims 15-16 and 21 lack novelty under PCT Article 33(2) as being anticipated by US 5,073,627 (CURTIS et al.).

US 5,073,627 teaches a multimeric fusion protein comprising two or more members of the GH supergene family (GM-CSF and IL-3) joined with or without a peptide linker (see entire document, especially claim 1). In addition, a multimeric fusion protein wherein one of the members is GM-CSF is taught (e.g., claim 1).

3. Claim 2 and 24 lack novelty under PCT Article 33(2) as being anticipated by SHU et al.

SHU et al. teach a fusion protein comprising a first protein joined by a peptide linker to a fragment of an immunoglobulin domain, comprising the cytokine IL-2 linked via a GGGSGGG linker to the CH3 of an immunoglobulin heavy chain (see entire document, especially "Abstract". Purification of this dimeric Ig fusion protein is also taught (e.g. "Abstract" and Section 2.5).

4. Claims 15-16 and 21 lack novelty under PCT Article 33(2) as being anticipated by CURTIS et al.

CURTIS et al. teach a multimeric fusion protein comprising GM-CSF either linked directly or through a peptide linker to IL-3 (see entire document).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

5. Claim 2 lacks novelty under PCT Article 33(2) as being anticipated by US 5,723,125 (CHANG et al.).

US 5,723,125 teaches a fusion protein comprising a human interferon linked to an immunoglobulin Fc via a peptide linker comprising Gly and Ser (see entire document, e.g., "Abstract"). US 5,723,125 also teaches that the incorporation of a linker sequence is useful to avoid formation of neoantigens in the fusion protein (e.g., column 3, especially lines 22-35).

6. Claims 1-5 and 24 lack an inventive step under PCT Article 33(3) as being obvious over EITHER US 5,650,150 (GILLIES), OR SHU et al., OR US 5,723,125 in view of ROBINSON et al.

The claims are drawn to various peptide linker sequences used to link a first protein to an immunoglobulin domain.

US 5,650,150 has been discussed supra and teach a first protein linked to an immunoglobulin domain, either directly or with a linker comprising a proteolytic cleavage site, and the dimeric protein's purification.

SHU et al. or US 5,723,125 likewise have been discussed supra and teach a first protein linked via a peptide linker sequence to an immunoglobulin domain.

Neither US 5,650,150, SHU et al. nor US 5,723,125 teach the exact linkers recited.

ROBINSON et al. teach various linkers, and that different linkers comprising various amount and sequence compositions of Gly and Ser can be used for any given fusion construct in order to optimize the stability of single chain proteins (see entire document).

Given the teachings of the references in view of ROBINSON et al., it would have been obvious to the ordinary artisan at the time the invention was made to select for and optimize various linker sequences depending upon the first protein linked to the immunoglobulin domain. The ordinary artisan would have been motivated to optimize this linkage in order to obtain a stable fusion protein. Given the teachings of the references, the ordinary artisan would have had a reasonable expectation of success in producing any particular linkers comprising varying ratios and sequences of Gly and Ser. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

7. Claims 1-5, 15-21 and 24-25 appear to have industrial applicability as defined by PCT Article 33(4) since the fusion proteins of the instant invention could be used in the various methods of stimulating cell growth.

---------- NEW CITATIONS ----------

US 5,073,627 A (CURTIS et al.) 17 December 1997, (17-12-1997), see entire document, especially "Abstract" and "Claims".

US 5,650,150 A (GILLIES) 22 July 1997, (22-7-1997), see entire document, especially "Claims".

ROBINSON et al. Optimizing the stability of single-chain proteins by linker length and composition mutagenesis. Proc. Natl. Acad. Sci. USA. May 1998, Vol. 95, pages 5929-5934, see entire document, esecially "Discussion".

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19336

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 39/395, 39/00; C07K 16/001/00
US CL :424/134.1, 185.1, 192.1; 435/69.7; 530/387.3, 351,
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/134.1, 185.1, 192.1; 435/69.7; 530/387.3, 351,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

West, US Patent ful, STN via medline, biosis, caplus, embase. Search terms: fusion, hybrid, chimer?, immunolglobulin, cytokine, growth factor, epo, gm-csf, ifn-gamma, linker peptide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	SHU et al. Generation and characterization of a single-gene encoded single-chain immunoglobulin-interleukin-2 fusion protein. Immunotechnology. 1995, Vol. 1, Nos. 3-4, pages 231-241, especially see abstract and page 233, column 1.	1-6 ---- 24
X - Y	CURTIS et al. Enhanced hematopoietic activity of a human granulocyte macrophage colony stimulating factor-interleukin 3 fusion protein. Proceedings of the National Academy of Sciences USA. July 1991, Vol. 88, pages 5809-5813, see page 5809, column 2.	16-18, 21 ---------- 19,
X - Y	US 5,723,125 A (CHANG et al.) 03 March 1998 (02.03.98), see abstract and column 2, lines 34-44.	1-6, 24 -------- 25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
28 SEPTEMBER 2000

Date of mailing of the international search report
14 NOV 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
FOZIA HAMUD
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19336

Box I bservations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-14, 22, 23, 26-37
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19336

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98/38212 A2 (GENETICS INSTITUTE, INC.) 03 September 1998 (03.09.98), see entire document.	1-6, 15-21, 24-25

replaced by Article 34

Claims:

1. A fusion protein comprising a soluble protein directly joined to an immunogl bulin (lg) domain, wherein the soluble protein is a growth hormone, a cytokine that is not IL-10, or an active variant thereof.

2. A fusion protein comprising a first protein joined by a peptide linker to a fragment of an immunoglobulin domain, wherein said peptide linker is not AspProGlu or Ser and wherein the soluble protein is a growth factor or a cytokine.

3. The fusion protein of claim 2, wherein the peptide linker is SerGly.

4. The fusion protein of claim 2, wherein the peptide linker is $Ser(GlyGlySer)_n$, wherein n is 1 to 7.

5. The fusion protein of claim 2, wherein the peptide linker is Ser(GlyGlySer) or $Ser(GlyGlySer)_2$.

6. The fusion protein of claims 1-5, wherein the Ig domain is IgG-Fc, IgG-C_H or IgG-C_L.

7. The fusion protein of claims 1-6, wherein the soluble protein is a member of the growth hormone (GH) supergene family.

8. The fusion protein of claim 7, wherein the member is granulocyte-colony stimulating factor (G-CSF).

9. The fusion protein of claim 8, wherein the fusion protein has an EC_{50} of less than about 300 ng/ml.

10. The fusion protein of claim 8, wherein cysteine-17 of G-CSF is replaced by serine.

11. The fusion protein of claim 7, wherein the member has an EC_{50} of less than about 1,000 ng/ml.

12. The fusion protein of claim 11, wherein the member is EPO or GH.

13. The fusion protein of claim 7, wherein the member is alpha interferon having an IC_{50} of less than about 1,000 ng/ml.

14. The fusion protein of claim 7, wherein the member is beta interferon, gamma interferon, GM-CSF, IL-11, TPO, SCF or Flt3 ligand.

15. A multimeric fusion protein comprising two or more members of the GH supergene family joined without a peptide linker.

16. A multimeric fusion protein comprising two or more members of the GH supergene family joined by at least one peptide linker.

17. The multimeric fusion protein of claims 15-16, wherein the members are G-CSF.

18. The multimeric fusion protein of claim 17, wherein the multimeric fusion protein is a dimeric G-CSF fusion protein.

19. The multimeric fusion protein of claims 15-16, wherein the members are EPO.

20. The multimeric fusion protein f claim 19, wherein the multimeric fusion protein is a dimeric EPO fusion protein.

21. The multimeric fusion protein of claims 15-16, wherein the members are growth h rmone, alpha interferon, beta interferon, gamma interferon, GM-CSF, IL-11, TPO, SCF, Flt3 ligand or a combination thereof.

22. The fusion protein of claims 16-21, wherein the peptide linker is SerGly.

23. The fusion protein of claims 16-21, wherein the peptide linker is $Ser(GlyGlySer)_n$, wherein n is 1 to 7.

24. A purified dimeric Ig fusion protein that is essentially free of monomeric Ig fusion protein, wherein the dimeric Ig fusion protein comprises a growth factor, a cytokine or active variant thereof and an Ig domain.

25. The purified dimeric Ig fusion protein of claim 24, wherein the growth factor is G-CSF or EPO.

26. A method of producing the Ig fusion proteins of claims 1-25, comprising:

(a) transfecting or transforming a host cell with at least one nucleic acid encoding a growth factor, a cytokine or active variant thereof and an Ig domain;

(b) culturing the host cell; and

(c) harvesting the Ig fusion proteins expressed by the host cell.

27. The method of claim 26, wherein the nucleic acid further encodes a peptide linker.

28. A nucleic acid encoding the Ig fusion protein of claims 1-25.

29. A host cell transfected or transformed with the nucleic acid of claim 28 enabling the host cell to express the Ig fusion protein.

30. The host cell of claim 29, wherein the host cell is a eukaryotic cell.

31. The host cell of claim 30, wherein the eukaryotic cell is a mammalian cell.

32. A method of purifying the fusion protein of claims 1-25, comprising:

(a) obtaining a composition comprising the fusion protein; and

(b) isolating the fusion protein from contaminants by column chromatography.

33. The method of claim 32, wherein the fusion protein is isolated from contaminants by size chromatography.

34. A method of treating a condition treatable with a member of the GH supergene family, comprising administering an effective amount of the fusion protein of claims 1-25 to a patient in need thereof.

35. The method of claim 34, wherein the fusion protein is a G-CSF-Ig fusion protein and the condition is a deficiency of blood neutrophils.

36. The method of claim 34, wherein the fusion protein is an EPO-Ig fusion protein and the condition is a deficient hematocrit.

37. A pharmaceutical composition comprising the Ig fusion protein of claims 1-25 in a pharmaceutically acceptable carrier.